# Hemodynamic Abnormalities During Muscle Metaboreflex Activation in Type 2 Diabetes Mellitus Patients

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**RESEARCH ARTICLE**

# Hemodynamic abnormalities during muscle metaboreflex activation in patients with type 2 diabetes mellitus

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[1]*Sports Physiology Laboratory, Department of Medical Sciences and Public Health, University of Cagliari, Cagliari, Italy;* [2]*Cardiovascular Physiology Laboratory, Department of Clinical and Biological Science, University of Torino, Turin, Italy; and* [3]*Faculty of Physical Education and Sport Sciences, Hakim Sabzevari University, Sabzevar, Iran*

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**Roberto S, Milia R, Doneddu A, Pinna V, Palazzolo G, Serra S, Orrù A, Hosseini Kakhak SA, Ghiani G, Mulliri G, Pagliaro P, Crisafulli A.** Hemodynamic abnormalities during muscle metaboreflex activation in patients with type 2 diabetes mellitus. *J Appl Physiol* 126: 444–453, 2019. First published December 13, 2018; doi: 10.1152/japplphysiol.00794.2018.—Metaboreflex is a reflex triggered during exercise or postexercise muscle ischemia (PEMI) by metaboreceptor stimulation. Typical features of metaboreflex are increased cardiac output (CO) and blood pressure. Patients suffering from metabolic syndrome display hemodynamic abnormalities, with an exaggerated systemic vascular resistance (SVR) and reduced CO response during PEMI-induced metaboreflex. Whether patients with type 2 diabetes mellitus (DM2) have similar hemodynamic abnormalities is unknown. Here we contrast the hemodynamic response to PEMI in 14 patients suffering from DM2 (age 62.7 ± 8.3 yr) and in 15 age-matched controls (CTLs). All participants underwent a control exercise recovery reference test and a PEMI test to obtain the metaboreflex response. Central hemodynamics were evaluated by unbiased operator-independent impedance cardiography. Although the blood pressure response to PEMI was not significantly different between the groups, we found that the SVR and CO responses were reversed in patients with DM2 as compared with the CTLs (SVR: 392.5 ± 549.6 and −14.8 ± 258.9 dyn·s$^{-1}$·cm$^{-5}$; CO: −0.25 ± 0.63 and 0.46 ± 0.50 l/m, respectively, in DM2 and in CTL groups, respectively; $P < 0.05$ for both). Of note, stroke volume (SV) increased during PEMI in the CTL group only. Failure to increase SV and CO was the consequence of reduced venous return, impaired cardiac performance, and augmented afterload in patients with DM2. We conclude that patients with DM2 have an exaggerated vasoconstriction in response to metaboreflex activation not accompanied by a concomitant increase in heart performance. Therefore, in these patients, blood pressure response to the metaboreflex relies more on SVR increases rather than on increases in SV and CO.

**NEW & NOTEWORTHY** The main new finding of the present investigation is that subjects with type 2 diabetes mellitus have an exaggerated vasoconstriction in response to metaboreflex activation. In these patients, blood pressure response to the metaboreflex relies more on systemic vascular resistance than on cardiac output increments.

blood pressure; cardiac preload; cardiovascular regulation; myocardial contractility; stroke volume

## INTRODUCTION

In normal subjects, the activation of the muscle metaboreflex leads to certain hemodynamic adjustments, which are characterized by elevation in mean arterial blood pressure (MBP) because of increments in both systemic vascular resistance (SVR) and cardiac output (CO) (1, 4, 9, 21, 32, 37, 40, 41, 54, 58, 59).

The postexercise muscle ischemia (PEMI) method is particularly useful to investigate hemodynamic dysregulation during metaboreflex in patients suffering from different cardiovascular as well as metabolic disorders. This is because during PEMI, the heart rate (HR) does not usually take part in the hemodynamic response (12, 22, 39), and cardiovascular adjustment relies only on changes in cardiac preload and inotropism, which maintain or increase stroke volume (SV), notwithstanding the augmented afterload (12). Thus, the PEMI method appears particularly useful to reveal hemodynamic abnormalities when impairments in cardiac preload and/or inotropism are present, as CO cannot be increased by recruiting the HR reserve.

In the several last years, studies conducted in humans have reported that, in healthiness, the normal hemodynamic response during PEMI is characterized mainly by a flow-mediated (i.e., CO increase) rather than a vasoconstrict-mediated (i.e., SVR increase) mechanism. Conversely, when cardiac preload and/or inotropism cannot be enhanced, then SV and CO cannot properly increase in response to PEMI. This, in turn, is accompanied by exaggerated SVR increments (7, 8, 10, 11, 29, 34, 35, 49). Moreover, it has been observed that in clinical conditions characterized by excessive sympathetic tone, such as hypertension, heart failure, obesity, and metabolic syndrome, there is a pronounced arteriolar constriction that causes exaggerated SVR increments, thereby leading to ventricular afterload elevation in response to the metaboreflex (6, 13, 17, 35, 45). This phenomenon limits ventricular pumping and reduces SV during the metaboreflex. Therefore, in this case, the exaggerated vasoconstriction is not the consequence of deficits in inotropism and/or preload. Rather, it is because of an elevated sympathetic tone per se.

Several evidences suggest that, similarly to what was observed for metabolic syndrome in type 2 diabetes mellitus (DM2), there is an exaggerated sympathetic tone during exercise and during the metaboreflex, with elevated vasoconstric-

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tion and blunted vasodilator response (18, 27, 49, 62). This is a type of response that is also observed in a number of other cardiovascular diseases, thus supporting the point of view that DM2 can be considered a cardiovascular disease (5, 52).

Since DM2 is often preceded by metabolic syndrome (14, 48), it can be hypothesized that similar hemodynamic dysregulation previously observed for metabolic syndrome during the metaboreflex obtained by PEMI [reduction in CO and elevated SVR (35)] is also present in patients with DM2. This may be of clinical importance also taking into consideration that patients with DM2 may experience autonomic dysfunction even worse compared with patients with metabolic syndrome (23). This would induce an exaggerated SVR increment during PEMI. Given the great social burden and the high incidence of cardiovascular complications related to DM2 (60), it would be of interest to ascertain whether the hemodynamic response during the PEMI-induced metaboreflex relies more on SVR or on CO in patients with DM2. This may be useful to develop strategies able to counteract the cardiovascular consequences of DM2. Surprisingly, very few studies have, to date, investigated hemodynamics during the metaboreflex activation in patients suffering from DM2. The only investigation we could find focused on HR, MBP, and sympathetic activity but did not take into consideration other cardiovascular parameters such as SV, CO, myocardial contractility, and cardiac preload (18).

This study was devised to assess cardiovascular responses to muscle metaboreflex activation in individuals suffering from DM2. To this aim, hemodynamic responses to PEMI were studied in a group of patients suffering from DM2 and in an age-matched control group of healthy individuals.

## METHODS

*Study population.* Two groups of subjects were recruited. The first group consisted of patients with a diagnosis of DM2. They were recruited on the basis of the following criteria: clinical history of DM2 for at least 5 yr, with stable metabolic condition [hemoglobin A1c (HbA1c) level $<9\%$ at the time of the study]; age $\leq 18$ yr and $\geq 70$ yr; absence of associated medical conditions that could interfere with the autonomic function and/or chronic cardiopulmonary diseases; and presence of signs or symptoms of peripheral neuropathy. Smokers and patients taking β-blockers, sympathomimetics, and/or tricyclic antidepressants were also excluded. After the screening process was completed, 14 patients (4 females) with diagnosis of DM2 were enrolled. Time since diagnosis was, on average (mean ± SD) 14.14 ± 5.48 yr. The mean ± SD age was 62.7 ± 8.3 yr, whereas the mean ± SD height, body mass, and body mass index (BMI) were 166.9 ± 6.4 cm, 79.8 ± 12.8 kg, and 28.6 ± 4.1 kg/m$^2$, respectively. All patients were on medication with oral hypoglycemic agents and 11 with insulin. Seven participants were also on medication for high blood pressure (3 with sartans and 4 with angiotensin-converting enzyme inhibitors) and eight for high cholesterol (statins).

The second group was a control (CTL) group. Age-matched, healthy subjects ($n = 15$; 4 women, mean ± SD age of 62.5 ± 8.2 yr) who were unaffected by any metabolic disease as resulting from anamnesis and physical examination agreed to participate in the present investigation. The mean ± SD height, body mass, and BMI were 163.3 ± 6.4 cm, 65.9 ± 8.4 kg, and 23.8 ± 2.7 kg/m$^2$, respectively.

All participants were sedentary. All subjects signed an informed consent after being fully informed about the experimental procedures. The study was approved by the local ethical committee and conforms to the declaration of Helsinki.

After enrollment, all the participants underwent a medical examination with anamnesis, ECG, and blood pressure. Blood samples were drawn for fasting glucose level and Hb1Ac measurement. After this preliminary medical screening, all subjects performed a cardiopulmonary exercise test (CPT) on an electromagnetically braked cycle ergometer (CUSTO Med, Ottobrunn, Germany) to assess their physical capacity. The CPT test consisted of a linear increase of workload (10 W/min), starting at 10 W at a pedaling frequency of 60 revolutions/min until exhaustion, which was taken as the point at which the subject experienced fatigue (i.e., unable to maintain a pedaling rate of at least 50 revolutions/min). Achievement of $\dot{V}O_{2max}$ was considered as the attainment of at least 2 of the following criteria: *1*) a plateau in $\dot{V}O_2$ despite increasing workload ($<80$ ml/min), *2*) respiratory exchange ratio above 1.10, and *3*) HR ± 10 beats/min of predicted maximum HR, calculated as 220 − age (19).

*Experimental design.* After the CPT test (the interval was at least 3 days, range 5–10 days), each subject was randomly assigned to the following study protocols to study metaboreflex activity.

The first study protocol was a PEMI session, which consisted of 3 min of resting, followed by 3 min of rhythmic (30 compressions/min) dynamic handgrip at 30% of the maximum, assessed as the peak reached during 5 previous maximal compressions on a hydraulic dynamometer (MAP 1.1, Kern, Balingen, Germany). Exercise was followed by 3 min of PEMI on the exercised arm, induced by rapidly (in $<3$ s) inflating an upper arm biceps cuff to 50 mmHg above peak exercise systolic pressure. The cuff was kept inflated for 3 min. Three minutes of recovery were further allowed after the cuff was deflated, for a total of 6 min of recovery. This protocol has been demonstrated to be capable to trap the muscle metabolites in the exercising limb and to maintain stimulation of the metaboreceptors, and it has previously been employed in similar experimental settings (11, 31, 50).

The second study protocol was a control exercise recovery (CER) session. The same rest-exercise protocol used for PEMI was employed, but handgrip was followed by a control exercise recovery of 6 min without cuff inflation.

PEMI and CER sessions were spaced by at least 2 days (interval of 2–6 days). All experiments were carried out in a temperature-controlled, air-conditioned room (temperature set at 22°C and relative humidity 50%).

Subjects were asked to refrain from consuming alcoholic and caffeinated beverages for 12 h before scheduled experiments. All enrolled women were in menopause as self-reported.

*Hemodynamic measurements.* Throughout the PEMI and the CER tests, hemodynamic parameters were measured by impedance cardiography (NCCOM 3, BoMed, Irvine, CA). This method allows continuous noninvasive collecting of thoracic electrical impedance (Z0) variations, which can be digitized by using a chart recorder (ADInstruments, PowerLab 8sp, Castle Hill, Australia) and then analyzed offline. Changes in Z0 are representative of the pulsatile aortic blood flow because of ventricular systole, which causes a proportional fluctuation in electrical conductivity. Therefore, it is assumed that changes in Z0 during systole are representative of changes in SV. The Sramek-Bernstein equation was employed to measure SV (3).

This procedure has been previously used in our laboratory during similar investigations aiming to study hemodynamics during metaboreflex in normal subjects and in various cardiovascular and metabolic diseases. A detailed description of the data acquisition and processing methods are present in these previous papers (7, 10, 29, 34, 35, 49). Briefly, NCCOM 3-derived analog traces of electrocardiogram, Z0, and Z0 first derivative were stored, and SV was calculated offline along with the pre-ejection period (PEP) and left ventricular ejection time (VET) (31, 55). Diastolic time was obtained by subtracting the sum of PEP and VET from the cardiac cycle's total period. The ventricular filling rate (VFR), a measure of the mean rate of diastolic blood flux, was calculated by dividing SV by diastolic time (8, 16, 31, 55). The mean ventricular emptying rate (VER) was calculated as

SV-to-VET ratio and was considered an index of myocardial performance (16, 54). HR was assessed as the reciprocal of the electrocardiogram R-R interval, and CO was obtained by multiplying SV by HR. Systolic and diastolic blood pressure were measured every minute by the same physician throughout all protocol sessions using a standard manual sphygmomanometer placed in the nonexercised arm. MBP was calculated by using formulae, which took into account changes in the diastolic and systolic periods because of changes in HR (36, 53). SVR was obtained by multiplying the MBP-to-CO ratio by 80, where 80 is a conversion factor to change units to standard resistance units.

To obtain an index of sympathetic activity toward the left ventricle, the measure of PEP was employed. This parameter has been demonstrated to be a useful index of cardiac sympathetic activity, with negligible influence of parasympathetic tone. Indeed, PEP shortening is the result of a more rapid development of force and intraventricular pressure, which are mostly the result of changes in sympathetic activity, as ventricles are not innervated by parasympathetic hormones. Moreover, PEP is not substantially altered by changes in HR (33), but it is sensitive to changes in cardiac preload, afterload, and contractility.

*Data analysis and calculation.* Data are presented as means ± SD. The Kolmogorov-Smirnov test was used to assess the normality of distribution for each variable. Parametric tests were used for variables with normal distribution. To calculate the necessary sample size, we performed a power calculation using a power of 85%, an overall type 1 error of 0.05 (two-sided), and a 20% difference between groups in the studied variables. Twelve subjects were needed to obtain an adequate statistical power. Differences between groups in anthropometric characteristics and physical capacity assessed during the CPT test were found out by unpaired *t*-test. Hemodynamic data gathered during the PEMI and the CER tests were averaged over 1 min. Differences between groups in absolute values at the third minute of rest, at the third minute of exercise, and at the third minute of recovery were carried out by the two-way ANOVA (factors: group and condition) followed by Bonferroni post hoc when appropriate. Furthermore, for each parameter, the difference between values of the PEMI and the CER tests during the third minute of recovery (i.e., when a steady-state in parameters was supposed to be reached) was calculated. This allowed us to assess responses because of metaboreflex activity (11, 29, 34). The difference between groups in parameters' responses was carried out by unpaired *t*-test. Statistical analysis was carried out by employing commercially available software (GraphPad Prism). Statistical significance was established as a $P$ value of $<0.05$ in all cases.

## RESULTS

The protocol was completed by all subjects. The Kolmogorov-Smirnov test confirmed the normal distribution for all the parameters examined. None of the participants reported unbearable pain or discomfort during the periods of PEMI. Subjects of the DM2 group were similar in age ($P = 0.9485$) and height ($P = 0.1126$), whereas they exhibited higher values of body mass ($P = 0.0017$) and BMI ($P = 0.0009$) in comparison with the CTL subjects. Patients with DM2 showed higher values of fasting glucose (106.41 ± 11.2 vs. 92.30 ± 4.6 mg/dl, $P < 0.0001$) and higher levels of HbA1c (7.05 ± 0.10% vs. 4.80 ± 0.12%, $P < 0.0001$) than CTL subjects. Results of the CPT demonstrated that CTL subjects reached higher levels of maximum HR (158.5 ± 6.8 vs. 131.5 ± 14.6 beats/min, $P < 0.0001$) and $\dot{V}O_{2max}$ (28.1 ± 3.5 vs. 20.2 ± 4.2 ml·min$^{-1}$·kg$^{-1}$, $P < 0.0001$) as compared with the DM2 group.

The values of data recorded during rest periods preceding hand-grip strains are reported in Table 1. Statistics revealed that SV and CO were, on average, lower in the DM2 group than in the CTL group, whereas SVR and PEP were higher. The other parameters were unaffected by group. Condition (i.e., PEMI or CER test) did not affect the variables' level, and there was no significant interaction effect.

Table 2 shows values of parameters gathered at the third minute of handgrip. Statistics showed that SV, CO, VFR, and VER were lower in the DM2 group than in the CTL group, whereas SVR and PEP were higher. Condition did not influence any variable, and there was no significant interaction effect.

Figures 1–3 show hemodynamic variables reported as absolute values during the PEMI and CER test and as their responses (i.e., the difference between PEMI and CER tests). Figure 1*A* shows that, on average, HR was higher in the DM2 group than in the CTL group, whereas Fig. 1*B* illustrates that there was no difference between groups in HR response, i.e., the response because of metaboreflex activity. SV (Fig. 1*C*) was higher in the CTL group in comparison with the DM2 group. Moreover, the response of this parameter was higher in the CTL group as compared with the DM2 group (Fig. 1*D*).

Table 1. *Hemodynamic data values during resting period of the PEMI and CER tests in DM2 and CTL groups*

| | DM2 | CTL | *P* Value Condition Effect | *P* Value Group Effect | *P* Value Interaction Effect |
|---|---|---|---|---|---|
| HR, beats/min | PEMI 70.8 ± 11.7 | PEMI 67.2 ± 9.7 | 1.0000 | 0.1458 | 0.8832 |
| | CER 71.2 ± 11.6 | CER 66.8 ± 7.3 | | | |
| SV, ml | PEMI 42.6 ± 6.7 | PEMI 63.7 ± 25.1 | 0.5356 | 0.0004 | 0.8896 |
| | CER 46.7 ± 8.9 | CER 66.3 ± 29.5 | | | |
| CO, l/min | PEMI 3 ± 0.72 | PEMI 4.1 ± 1.1 | 0.3990 | 0.0004 | 0.7876 |
| | CER 3.3 ± 0.8 | CER 4.3 ± 1.3 | | | |
| VFR, ml/s | PEMI 96.6 ± 30.8 | PEMI 135.2 ± 34.4 | 0.6518 | 0.0841 | 0.9258 |
| | CER 108 ± 38.5 | CER 142.7 ± 44.2 | | | |
| VER, ml/s | PEMI 177.5 ± 33.7 | PEMI 208.1 ± 68.4 | 0.5612 | 0.0972 | 0.7400 |
| | CER 191.4 ± 38.8 | CER 211.9 ± 75.3 | | | |
| MBP, mmHg | PEMI 95.9 ± 8.6 | PEMI 95.5 ± 12.9 | 0.6713 | 0.8874 | 1.0000 |
| | CER 97.1 ± 9.1 | CER 96.7 ± 11.4 | | | |
| SVR, dyn·s$^{-1}$·cm$^{-5}$ | PEMI 2,687.1 ± 766.4 | PEMI 1,982.2 ± 609.3 | 0.6213 | 0.0023 | 0.5807 |
| | CER 2,489.3 ± 733.1 | CER 1,993.3 ± 746.6 | | | |
| PEP, ms | PEMI 151.4 ± 22.8 | PEMI 133.5 ± 18.6 | 0.7007 | 0.0045 | 0.9804 |
| | CER 149.2 ± 30.1 | CER 131.0 ± 19.9 | | | |

Values are means ± SD. CER, control exercise recovery; CO, cardiac output; CTL, control; DM2, type 2 diabetes mellitus; HR, heart rate; MBP, mean arterial blood pressure; PEMI, postexercise muscle ischemia; PEP, pre-ejection period; SV, stroke volume; SVR, systemic vascular resistance; VER, ventricular emptying rate; VFR, ventricular filling rate.

Table 2. *Hemodynamic data values at the third minute of exercise during PEMI and CER tests in DM2 and CTL groups*

| | DM2 | CTL | *P* Value Condition Effect | *P* Value Group Effect | *P* Value Interaction Effect |
|---|---|---|---|---|---|
| HR, beats/min | PEMI 77.7 ± 12.4 | PEMI 80.5 ± 13.4 | 0.9093 | 0.5702 | 0.4277 |
| | CER 78.4 ± 11.6 | CER 76.3 ± 9.05 | | | |
| SV, ml | PEMI 47.9 ± 8.6 | PEMI 72.3 ± 32.6 | 0.8367 | 0.0002 | 0.7008 |
| | CER 46.7 ± 9.8 | CER 76.3 ± 36.2 | | | |
| CO, l/min | PEMI 3.7 ± 0.87 | PEMI 5.53 ± 2 | 0.9335 | <0.0001 | 0.9337 |
| | CER 3.7 ± 0.86 | CER 5.6 ± 2.1 | | | |
| VFR, ml/s | PEMI 127.7 ± 38.1 | PEMI 218.5 ± 98.9 | 0.8546 | <0.0001 | 0.8856 |
| | CER 134.3 ± 54.7 | CER 219.3 ± 92.2 | | | |
| VER, ml/s | PEMI 190 ± 36.7 | PEMI 233.5 ± 89.1 | 0.9001 | 0.0104 | 0.8220 |
| | CER 188.2 ± 30.9 | CER 239.8 ± 88.6 | | | |
| MBP, mmHg | PEMI 116.9 ± 10.1 | PEMI 117.9 ± 15.5 | 0.9154 | 0.5970 | 0.7914 |
| | CER 115.5 ± 12.7 | CER 118.5 ± 17.5 | | | |
| SVR, $dyn \cdot s^{-1} \cdot cm^{-5}$ | PEMI 2,626.8 ± 634.8 | PEMI 1,887.7 ± 580.6 | 0.7463 | <0.0001 | 0.9858 |
| | CER 2,579.4 ± 572.8 | CER 1,834.8 ± 560.2 | | | |
| PEP, ms | PEMI 133.6 ± 25.6 | PEMI 121.4 ± 24.3 | 0.9578 | 0.0446 | 0.8502 |
| | CER 135.2 ± 27.0 | CER 120.5 ± 23.4 | | | |

Values are means ± SD. CER, control exercise recovery; CO, cardiac output; CTL, control; DM2, type 2 diabetes mellitus; HR, heart rate; MBP, mean arterial blood pressure; PEMI, postexercise muscle ischemia; PEP, pre-ejection period; SV, stroke volume; SVR, systemic vascular resistance; VER, ventricular emptying rate; VFR, ventricular filling rate.

Similarly, CO was higher in the CTL group, both in terms of absolute values and in its response (Fig. 1, *E* and *F*, respectively).

Figure 2, *A* and *C* demonstrates that both VFR and VER absolute values, as well as their responses (Fig. 2, *B* and *D*), were higher in the CTL group than in the DM2 group.

Condition (i.e., PEMI and CER test) significantly affected MBP, which was higher during the PEMI as compared with the CER test, whereas group did not lead to any significant difference (Fig. 3*A*). The response in MBP was not different between groups (Fig. 3*B*). SVR was, on average, higher in the DM2 than in the CTL group (Fig. 3*C*), whereas the response in this parameter was lower in the CTL group than in DM2 group (Fig. 3*D*). Finally, absolute values of PEP were significantly higher in the DM2 group than in the CTL group, whereas its response was similar (Fig. 3, *E* and *F*, respectively).

## DISCUSSION

The present study aimed at discovering whether the hemodynamic response during the metaboreflex elicited by PEMI relied more on SVR than on CO in patients with DM2. To this end, central hemodynamics during PEMI were studied in a group of patients suffering from DM2 and in an age-matched control group of healthy individuals. We found that patients with DM2 exaggeratedly increased SVR (i.e., increased arteriolar vasoconstriction) in response to the metaboreflex activation as compared with healthy controls, whereas SV and CO response were blunted. In these patients, blood pressure response to the metaboreflex relies more on SVR increases rather than on cardiac performance adaptations.

Results show that patients with DM2 had higher SVR levels already at rest in comparison with the CTL group. This higher level of SVR was maintained during dynamic handgrip and during the PEMI maneuver utilized to isolate the metaboreflex. Moreover, the SVR increments because of metaboreflex activity were higher in the DM2 group than in the CTL group. These results closely resemble what was recently observed in patients with metabolic syndrome (35) and support the concept that in patients suffering from metabolic disorders such as obesity, metabolic syndrome, and DM2, exaggerated arterial vasoconstriction and reduced vasodilator responsiveness are present during exercise and during metaboreflex stimulation (15, 26, 61, 62).

Although subjects of the CTL group increased CO during PEMI, patients with DM2 were unable to increase CO during the metaboreflex. The failure to increase CO during PEMI was not due to a different chronotropic response. Actually, neither the DM2 nor the CTL group showed any HR increment during the PEMI session in comparison with the CER test. This is consistent with the view that HR does not take part in the hemodynamic response during PEMI (12, 22, 39). As a consequence, in both groups, the blood pressure increment during the metaboreflex could rely only on the reserves in preload/SV and/or in SVR/afterload.

The incapacity of patients with DM2 to increase CO was due to their inability to enhance SV. Several factors could explain this phenomenon. First, it may be that patients with DM2 had an impaired myocardial contractile response. This possibility is supported by VER, which can be considered an index of myocardial performance. VER was lower in patients with DM2 with respect to the CTL group already at rest, and it kept lower levels during the metaboreflex activation. Moreover, during PEMI, there was no detectable VER response in patients with DM2, whereas VER augmented in CTL subjects. To further support the presence of a reduced myocardial performance, there was the PEP behavior, which was longer in patients with DM2 at rest, during exercise, and during PEMI in comparison with the CTL group. PEP is related to the rate of development of force and intraventricular pressure. Increase in myocardial contractility is accompanied by PEP shortening, whereas impairments and reductions in cardiac performance cause PEP lengthening (28, 33). A second reason that can explain why patients with DM2 could not increase SV during the metaboreflex can be directly related to the pronounced arteriolar constriction (i.e., SVR increment) they experienced during PEMI. In normal individuals with an intact contractility reserve, an increase in afterload is normally counteracted by an enhancement in myocardial performance, which in turn maintains SV (i.e., the Anrep phenomenon). However, this was not the case

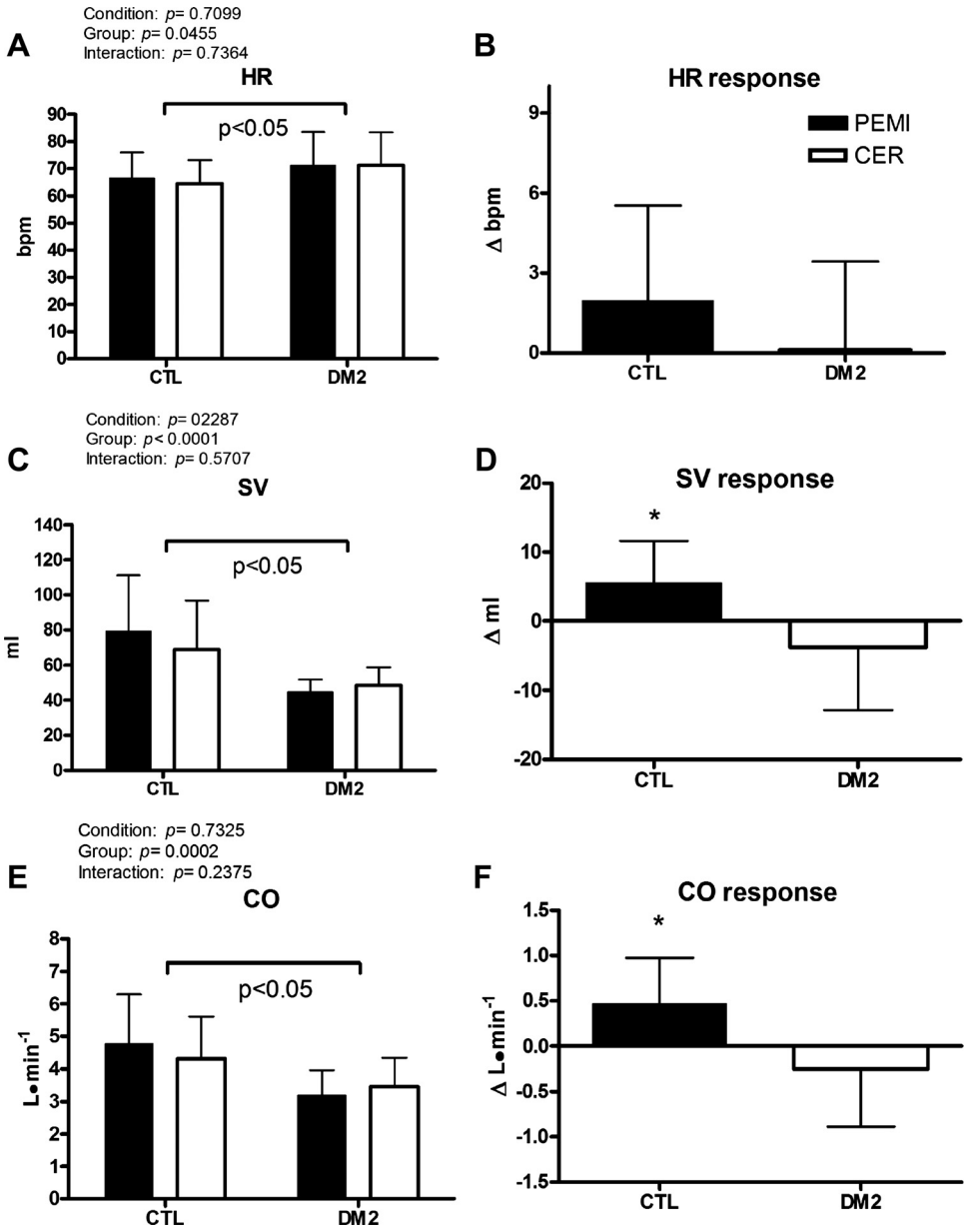


Fig. 1. Absolute values during postexercise muscle ischemia (PEMI) and control exercise recovery (CER) tests and response in heart rate (HR; *A* and *B*), stroke volume (SV; *C* and *D*), and cardiac output (CO; *E* and *F*) in type 2 diabetes mellitus (DM2) and control (CTL) groups. Values are mean ± SD. A horizontal bracket indicates the overall main effect of groups. Δ, change in; bpm, beats per minute. $*P < 0.05$ vs. DM2 group.

in patients with DM2. It is therefore possible that these patients suffer from systolic impairment, which was revealed by the PEMI-induced SVR increment. A further explanation for the lack of SV response in patients with DM2 may be the reduced venous return. From recent and early findings, it appears that the capacity to centralize blood volume and to increase venous return may be important in normal hemodynamics during metaboreflex (2, 8, 34, 51, 57, 58). In the present investigation, VFR was employed as a measure of venous return. The VFR level was lower in the DM2 group than in the CTL group during both exercise and PEMI. Furthermore, during PEMI, the response of this parameter was lower than in the CTL group, thereby suggesting a reduction in diastolic flux toward the heart in the DM2 group. The lower VFR level and its reduced response during the metaboreflex could be the consequence of a diastolic dysfunction in patients with DM2. Some recent studies found abnormalities in left ventricular relaxation in patients with metabolic syndrome (30, 34, 47), even though the observed abnormal diastolic function is not unanimously reported (56). The impaired diastolic functions could also prevent the normal recruitment of the Frank-Starling mechanism, and this provides a further pathophysiological basis to explain the reduced capacity to enhance cardiac performance in these patients.

Another phenomenon that could account for the impaired VFR was the reduction in circulating blood volume, which has already been reported in patients with DM2 (27, 49). It is, however, to be acknowledged that we did not assess blood volume. Thus, this remains speculative.

Nevertheless, it is likely that a combination of reduced myocardial performance, reduced preload, and increased afterload all concurred in reducing SV in patients with DM2. In particular, the presence of a subclinical contractility and diastolic impairments cannot be excluded, even though patients in

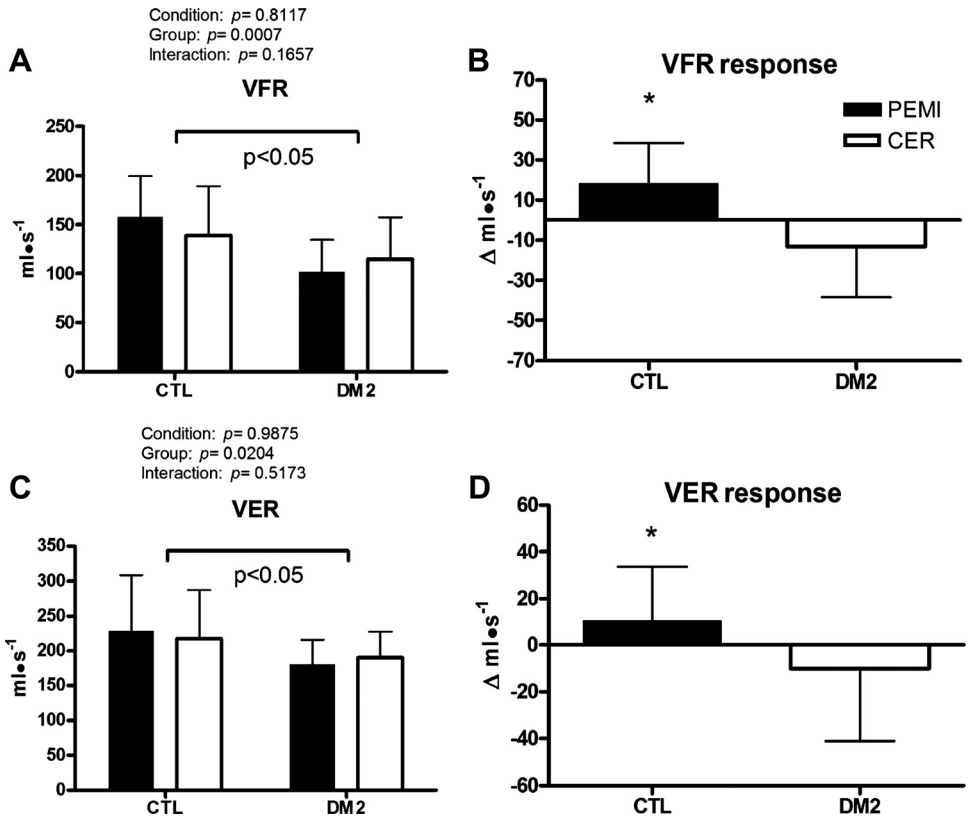


Fig. 2. Absolute values during postexercise muscle ischemia (PEMI) and control exercise recovery (CER) tests and response in ventricular filling rate (VFR; *A* and *B*) and ventricular emptying rate (VER; *C* and *D*) in type 2 diabetes mellitus (DM2) and control (CTL) groups. Values are mean ± SD. A horizontal bracket indicates the overall main effect of groups. Δ, change in. $^*P < 0.05$ vs. DM2 group.

the DM2 group did not show any sign or symptoms of overt heart failure. It cannot be excluded that the PEMI could detect early signs of systolic and diastolic impairment before the development of overt signs of heart failure. Results of the present investigation confirm that in healthy individuals, during the metaboreflex obtained by PEMI, a central role in the metaboreflex-induced blood pressure response is played by the ability to maintain or increase CO. In contrast, the recruitment of the afterload reserve (i.e., arteriolar vasoconstriction) appears to become more important in clinical situations when CO cannot be properly adjusted, such as in systolic and diastolic heart failure, coronary artery disease, and aging (10, 29, 34, 42, 43, 51). In all these conditions, the target blood pressure is achieved mainly by an SVR-mediated, rather than by a flow-mediated, mechanism.

Another finding of the present study is that patients with DM2 showed reduced levels of $\dot{V}O_{2max}$ during the CPT in comparison with CTL subjects. In detail, their $\dot{V}O_{2max}$ level was ~70% of that reached by subjects of the CTL group. According to the Weber classification of heart failure, the average $\dot{V}O_{2max}$ of the DM2 group (i.e., 20.21 ± 4.20 ml·min$^{-1}$·kg$^{-1}$) was close to the cutoff of 20 ml·min$^{-1}$·kg$^{-1}$ to be included in the mild-moderate stage of deterioration of functional capacity. In particular, 7 of the 14 patients were below, whereas 7 were above the cutoff (range: 14.17–25.47 ml·min$^{-1}$·kg$^{-1}$). This indicates that, in terms of aerobic capacity, half of the patients of the DM2 group could be already classified as in the mild-moderate stage of deterioration of functional capacity, and this supports the notion that some degree of exercise intolerance was present in patients with DM2 (46). This finding is not novel since altered exercise tolerance has been described several times in the recent past (46). We cannot provide any definitive explanation for the altered exercise capacity in patients with DM2, which was beyond the scope of the present investigation. One possible explanation could be the reduced $HR_{max}$ reached by these patients in comparison with the CTL group (see results), which suggests the presence of an increased level of perceived exertion and/or chronotropic incompetence.

One interesting phenomenon was that, notwithstanding the sustained SVR, MBP was similar between groups, both in terms of absolute value and in terms of MBP response, despite the fact that 7 out of 14 patients were receiving treatment for high blood pressure. It is likely that their therapy was effective in controlling their blood pressure level.

It remains to be established what caused the elevated SVR. One possibility was the occurrence of sympathetic overdrive which led to exaggerated arteriolar constriction. The presence of increased sympathetic tone during exercise in DM2 was observed by several investigations in the past (26, 62). The consensus is that hyperinsulinemia and insulin resistance associated with DM2 lead to sympathetic overactivation (20, 49). However, data of the present investigation does not highlight any difference in sympathetic response between the two groups studied, as PEP response was almost identical between the groups. It should, however, be acknowledged that sympathetic activity was not directly measured, and that PEP analysis is only a surrogate. Further research with direct sympathetic assessment is needed to better elucidate the role of the sympathetic system in cardiovascular dysregulation of individuals with DM2.

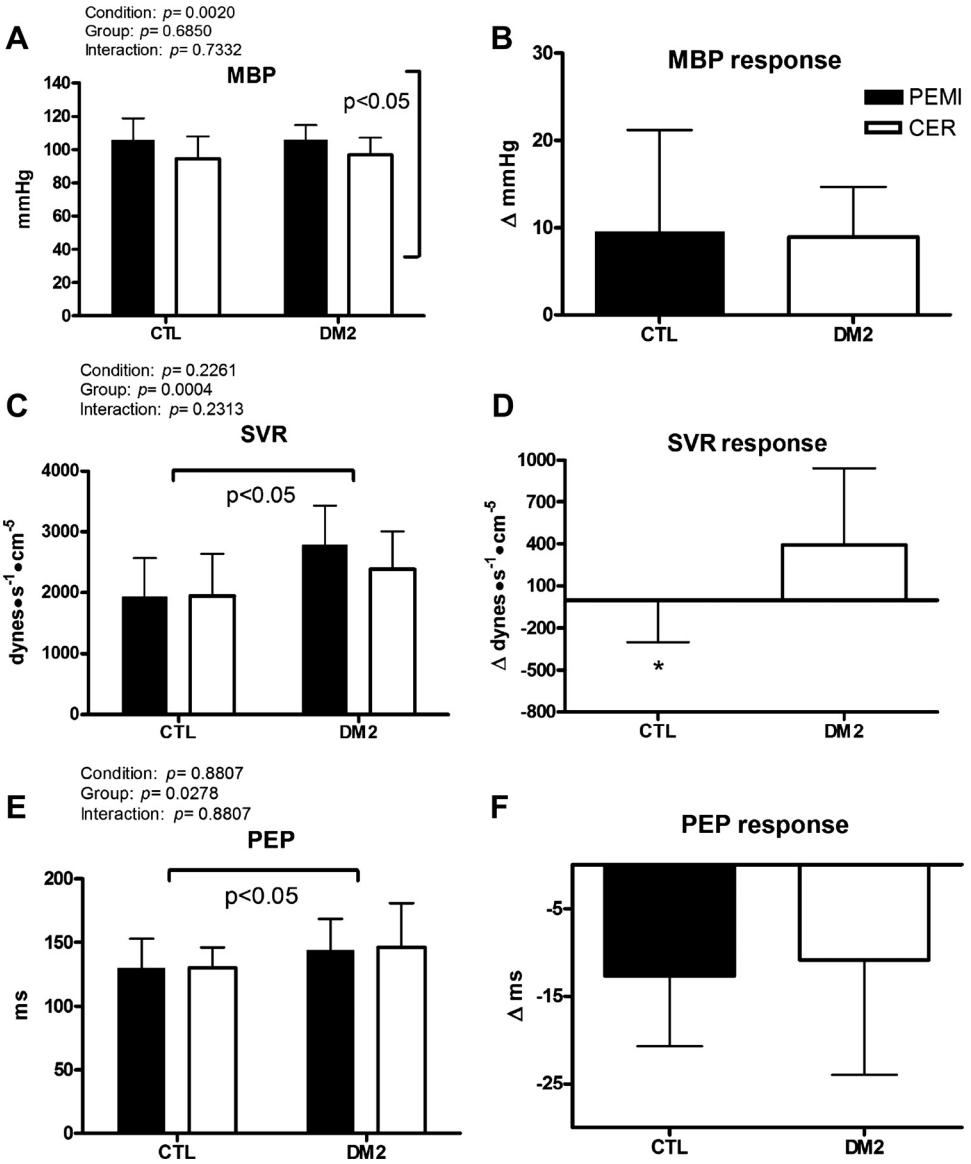


Fig. 3. Absolute values during postexercise muscle ischemia (PEMI) and control exercise recovery (CER) tests and response in mean arterial blood pressure (MBP; *A* and *B*), systemic vascular resistance (SVR; *C* and *D*), and pre-ejection period (PEP; *E* and *F*) in type 2 diabetes mellitus (DM2) and control (CTL) groups. Values are mean ± SD. A horizontal bracket indicates the overall main effect of groups. A vertical bracket denotes a main effect of condition. Δ, change in. $*P < 0.05$ vs. DM2 group.

Another potential contributor to the increased SVR in DM2 could be the presence of endothelial dysfunction (24, 26, 62). In health, the endothelium is a key factor in vascular homeostasis by producing a variety of vasodilators (e.g., NO and prostacyclin) and vasoconstrictor (i.e., angiotensin II, endothelin-1, thromboxane) substances. Moreover, the endothelium is important in local sympathetic restrains during exercise (i.e., the "functional sympatholysis"). In situations such as hyperglycemia and insulin resistance, the equilibrium between vasodilating and vasoconstricting products is altered, with abnormal vasomotor tone and reduced endothelium-mediated vasodilation in response to exercise (25, 26, 38, 44, 46). It also appears that endothelium-mediated functional sympatholysis is reduced in DM2 (46). Thus, an impaired endothelium function, with reduced capacity to vasodilate in response to the metaboreflex, could be responsible for the elevated SVR response observed in these patients.

An alternative explanation for the increased SVR may also have arisen from a compensatory phenomenon in response to the reduced CO associated with decreased SV. It could be hypothesized that mechanisms controlling the cardiovascular apparatus successfully counteracted the reduced CO by increasing SVR to maintain blood pressure.

*Limitations of the present study.* A potential limitation in the interpretation of our data is due to the fact that subjects of the DM2 group were on medication. Although individuals taking drugs known to affect autonomic nervous functions such as β-blockers, sympathomimetics, and/or tricyclic antidepressants were excluded, we cannot rule out that sartans, angiotensin-converting enzyme inhibitors, statins, oral hypoglycemic agents, and insulin could have affected the cardiovascular response to the PEMI maneuver in these patients. However, to the best of our knowledge, to date no studies have reported that any of these drugs had any impact on the hemodynamics during the metaboreflex.

Although static (isometric) causes a more pronounced metaboreflex activation and a more elevated blood pressure response than dynamic exercise, we preferred to use dynamic (rhythmic handgrip) instead of static effort, as this level of effort is more similar to everyday life activities. Our study did

not aim to investigate on hemodynamics during a high level of metaboreflex activation since patients only seldom perform isometric strains lasting for 2–3 min. Rather, we were interested in hemodynamics during a mild level of effort, an intensity is commonly reached during daily activities. We made this choice also considering that a high level of blood pressure could be dangerous in patients with DM2. Moreover, a number of previous published papers conducted in patients suffering from cardiac, metabolic, and neurological disorders employed similar protocols with dynamic exercise (8, 10, 11, 29, 34, 35, 50, 51). Thus, the present data can be compared with previous data of the cited studies.

*Physiological implication and clinical perspective.* Similar to what was found for some cardiovascular and metabolic diseases where CO cannot be properly augmented (10, 13, 29, 34, 35, 43, 51), in DM2, the target blood pressure during the metaboreflex relies on the afterload reserve (i.e., arteriolar vasoconstriction). This causes a functional shift from a CO to an SVR increase in the mechanism by which the cardiovascular system raises blood pressure in response to the metaboreflex. This phenomenon has been demonstrated to be detrimental in patients with heart failure, where excessive vasoconstriction at muscle level can initiate a vicious circle, which leads to exercise intolerance (45). Exaggerated increases in SVR during exercise are related to muscle hypoperfusion and, possibly, cerebral hypoperfusion. Both phenomena can contribute to the exercise intolerance often reported in patients with DM2 (45, 46, 61). Given the key contribution of the muscle metaboreflex to the blood pressure response to exercise, it would be clinically relevant to develop strategies able to counteract the augmented SVR during the metaboreflex. Future studies aiming to identify the mechanisms responsible to reduce such SVR hyper-response are needed. In the meantime, exercise training should be prescribed for DM2 since several observations suggest a protective effect of exercise training in these patients (44, 49).

In conclusion, the results of the present investigation provide evidence that patients suffering from DM2 have an abnormal hemodynamic response during metaboreflex, obtained by PEMI in comparison with healthy subjects. In these patients, the blood pressure response to the metaboreflex relies more on increments in SVR rather than on increases in CO. It appears that DM2 shifts the normal hemodynamic response from a predominantly flow-mediated to a vasoconstrict-mediated mechanism. This is similar to what is reported in metabolic syndrome and some cardiovascular diseases such as heart failure and hypertension. Results suggest that the incapacity to enhance SV plays a pivotal role in these altered hemodynamics and that this failure is the consequence of reduced venous return and/or impaired cardiac performance that cannot overcome the increase in afterload.

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## DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

## AUTHOR CONTRIBUTIONS

S.R., R.M., G.M., and A.C. conceived and designed research; S.R., R.M., A.D., V.P., G.P., S.S., A.O., S.A.H.K., G.G., G.M., P.P., and A.C. performed experiments; S.R., R.M., A.D., V.P., G.P., S.S., A.O., S.A.H.K., G.G., G.M., P.P., and A.C. analyzed data; S.R., R.M., A.D., G.G., P.P., and A.C. interpreted results of experiments; S.R., A.D., and A.C. prepared figures; S.R., R.M., A.D., V.P., G.P., A.O., S.A.H.K., G.G., G.M., P.P., and A.C. drafted manuscript; S.R., R.M., A.D., V.P., G.P., S.S., A.O., S.A.H.K., G.G., G.M., P.P., and A.C. edited and revised manuscript; S.R., R.M., A.D., V.P., G.P., S.S., A.O., S.A.H.K., G.G., G.M., P.P., and A.C. approved final version of manuscript.

## REFERENCES

1. **Amann M, Runnels S, Morgan DE, Trinity JD, Fjeldstad AS, Wray DW, Reese VR, Richardson RS.** On the contribution of group III and IV muscle afferents to the circulatory response to rhythmic exercise in humans. *J Physiol* 589: 3855–3866, 2011. doi:10.1113/jphysiol.2011.209353.
2. **Bastos BG, Williamson JW, Harrelson T, Nóbrega AC.** Left ventricular volumes and hemodynamic responses to postexercise ischemia in healthy humans. *Med Sci Sports Exerc* 32: 1114–1118, 2000. doi:10.1097/00005768-200006000-00012.
3. **Bernstein DP.** A new stroke volume equation for thoracic electrical bioimpedance: theory and rationale. *Crit Care Med* 14: 904–909, 1986. doi:10.1097/00003246-198610000-00017.
4. **Boushel R.** Muscle metaboreflex control of the circulation during exercise. *Acta Physiol (Oxf)* 199: 367–383, 2010. doi:10.1111/j.1748-1716.2010.02133.x.
5. **Candido R, Srivastava P, Cooper ME, Burrell LM.** Diabetes mellitus: a cardiovascular disease. *Curr Opin Investig Drugs* 4: 1088–1094, 2003.
6. **Choi H-M, Stebbins CL, Lee O-T, Nho H, Lee J-H, Chun J-M, Kim K-A, Kim J-K.** Augmentation of the exercise pressor reflex in prehypertension: roles of the muscle metaboreflex and mechanoreflex. *Appl Physiol Nutr Metab* 38: 209–215, 2013. doi:10.1139/apnm-2012-0143.
7. **Crisafulli A, Milia R, Lobina A, Caddeo M, Tocco F, Concu A, Melis F.** Haemodynamic effect of metaboreflex activation in men after running above and below the velocity of the anaerobic threshold. *Exp Physiol* 93: 447–457, 2008. doi:10.1113/expphysiol.2007.041863.
8. **Crisafulli A, Milia R, Vitelli S, Caddeo M, Tocco F, Melis F, Concu A.** Hemodynamic responses to metaboreflex activation: insights from spinal cord-injured humans. *Eur J Appl Physiol* 106: 525–533, 2009. doi:10.1007/s00421-009-1045-2.
9. **Crisafulli A, Piras F, Filippi M, Piredda C, Chiappori P, Melis F, Milia R, Tocco F, Concu A.** Role of heart rate and stroke volume during muscle metaboreflex-induced cardiac output increase: differences between activation during and after exercise. *J Physiol Sci* 61: 385–394, 2011. doi:10.1007/s12576-011-0163-x.
10. **Crisafulli A, Salis E, Tocco F, Melis F, Milia R, Pittau G, Caria MA, Solinas R, Meloni L, Pagliaro P, Concu A.** Impaired central hemodynamic response and exaggerated vasoconstriction during muscle metaboreflex activation in heart failure patients. *Am J Physiol Heart Circ Physiol* 292: H2988–H2996, 2007. doi:10.1152/ajpheart.00008.2007.
11. **Crisafulli A, Tocco F, Milia R, Angius L, Pinna M, Olla S, Roberto S, Marongiu E, Porcu M, Concu A.** Progressive improvement in hemodynamic response to muscle metaboreflex in heart transplant recipients. *J Appl Physiol (1985)* 114: 421–427, 2013. doi:10.1152/japplphysiol.01099.2012.
12. **Crisafulli A.** The impact of cardiovascular diseases on cardiovascular regulation during exercise in humans: studies on metaboreflex activation elicited by the post-exercise muscle ischemia method. *Curr Cardiol Rev* 13: 293–300, 2017. doi:10.2174/1573403X13666170804165928.
13. **Delaney EP, Greaney JL, Edwards DG, Rose WC, Fadel PJ, Farquhar WB.** Exaggerated sympathetic and pressor responses to handgrip exercise in older hypertensive humans: role of the muscle metaboreflex. *Am J Physiol Heart Circ Physiol* 299: H1318–H1327, 2010. doi:10.1152/ajpheart.00556.2010.
14. **Eckel RH, Grundy SM, Zimmet PZ.** The metabolic syndrome. *Lancet* 365: 1415–1428, 2005. doi:10.1016/S0140-6736(05)66378-7.
15. **Fournier SB, Reger BL, Donley DA, Bonner DE, Warden BE, Gharib W, Failinger CF, Olfert MD, Frisbee JC, Olfert IM, Chantler PD.** Exercise reveals impairments in left ventricular systolic function in patients with metabolic syndrome. *Exp Physiol* 99: 149–163, 2014. doi:10.1113/expphysiol.2013.075796.
16. **Gledhill N, Cox D, Jamnik R.** Endurance athletes' stroke volume does not plateau: major advantage is diastolic function. *Med Sci Sports Exerc* 26: 1116–1121, 1994. doi:10.1249/00005768-199409000-00008.

17. **Greaney JL, Matthews EL, Boggs ME, Edwards DG, Duncan RL, Farquhar WB.** Exaggerated exercise pressor reflex in adults with moderately elevated systolic blood pressure: role of purinergic receptors. *Am J Physiol Heart Circ Physiol* 306: H132–H141, 2014. doi:10.1152/ajpheart.00575.2013.
18. **Holwerda SW, Restaino RM, Manrique C, Lastra G, Fisher JP, Fadel PJ.** Augmented pressor and sympathetic responses to skeletal muscle metaboreflex activation in type 2 diabetes patients. *Am J Physiol Heart Circ Physiol* 310: H300–H309, 2016. doi:10.1152/ajpheart.00636.2015.
19. **Howley ET, Bassett DR Jr, Welch HG.** Criteria for maximal oxygen uptake: review and commentary. *Med Sci Sports Exerc* 27: 1292–1301, 1995. doi:10.1249/00005768-199509000-00009.
20. **Huggett RJ, Scott EM, Gilbey SG, Bannister J, Mackintosh AF, Mary DA.** Disparity of autonomic control in type 2 diabetes mellitus. *Diabetologia* 48: 172–179, 2005. doi:10.1007/s00125-004-1601-6.
21. **Ichinose MJ, Sala-Mercado JA, Coutsos M, Li Z, Ichinose TK, Dawe E, O'Leary DS.** Modulation of cardiac output alters the mechanisms of the muscle metaboreflex pressor response. *Am J Physiol Heart Circ Physiol* 298: H245–H250, 2010. doi:10.1152/ajpheart.00909.2009.
22. **Iellamo F, Pizzinelli P, Massaro M, Raimondi G, Peruzzi G, Legramante JM.** Muscle metaboreflex contribution to sinus node regulation during static exercise: insights from spectral analysis of heart rate variability. *Circulation* 100: 27–32, 1999. doi:10.1161/01.CIR.100.1.27.
23. **Istenes I, Körei AE, Putz Z, Németh N, Martos T, Keresztes K, Kempler MS, Erzsébet VO, Vargha P, Kempler P.** Heart rate variability is severely impaired among type 2 diabetic patients with hypertension. *Diabetes Metab Res Rev* 30: 305–312, 2014. doi:10.1002/dmrr.2496.
24. **Joyner WL, Mayhan WG, Johnson RL, Phares CK.** Microvascular alterations develop in Syrian hamsters after the induction of diabetes mellitus by streptozotocin. *Diabetes* 30: 93–100, 1981. doi:10.2337/diab.30.2.93.
25. **Kim JA, Montagnani M, Koh KK, Quon MJ.** Reciprocal relationships between insulin resistance and endothelial dysfunction: molecular and pathophysiological mechanisms. *Circulation* 113: 1888–1904, 2006. doi:10.1161/CIRCULATIONAHA.105.563213.
26. **Kingwell BA, Formosa M, Muhlmann M, Bradley SJ, McConell GK.** Type 2 diabetic individuals have impaired leg blood flow responses to exercise: role of endothelium-dependent vasodilation. *Diabetes Care* 26: 899–904, 2003. doi:10.2337/diacare.26.3.899.
27. **Lalande S, Hofman PL, Baldi JC.** Effect of reduced total blood volume on left ventricular volumes and kinetics in type 2 diabetes. *Acta Physiol (Oxf)* 199: 23–30, 2010. doi:10.1111/j.1748-1716.2010.02081.x.
28. **Lewis RP, Rittogers SE, Froester WF, Boudoulas H.** A critical review of the systolic time intervals. *Circulation* 56: 146–158, 1977. doi:10.1161/01.CIR.56.2.146.
29. **Magnani S, Roberto S, Sainas G, Milia R, Palazzolo G, Cugusi L, Pinna V, Doneddu A, Kakhak SAH, Tocco F, Mercuro G, Crisafulli A.** Metaboreflex-mediated hemodynamic abnormalities in individuals with coronary artery disease without overt signs or symptoms of heart failure. *Am J Physiol Heart Circ Physiol* 314: H452–H463, 2018. doi:10.1152/ajpheart.00436.2017.
30. **Mahmud A, Almuntaser I, Brown A, King G, Crean P, Feely J.** Left ventricular structural and functional changes in the metabolic syndrome. *J Cardiometab Syndr* 4: 81–88, 2009. doi:10.1111/j.1559-4572.2008.00043.x.
31. **Marongiu E, Piepoli M, Milia R, Angius L, Pinna M, Bassareo P, Roberto S, Tocco F, Concu A, Crisafulli A.** Effects of acute vasodilation on the hemodynamic response to muscle metaboreflex. *Am J Physiol Heart Circ Physiol* 305: H1387–H1396, 2013. doi:10.1152/ajpheart.00397.2013.
32. **McNulty CL, Moody WE, Wagenmakers AJ, Fisher JP.** Effect of muscle metaboreflex activation on central hemodynamics and cardiac function in humans. *Appl Physiol Nutr Metab* 39: 861–870, 2014. doi:10.1139/apnm-2013-0414.
33. **Michael S, Graham KS, Davis GM.** Cardiac autonomic responses during exercise and post-exercise recovery using heart rate variability and systolic time intervals-a review. *Front Physiol* 8: 301, 2017. doi:10.3389/fphys.2017.00301.
34. **Milia R, Roberto S, Mulliri G, Loi A, Marcelli M, Sainas G, Milia N, Marongiu E, Crisafulli A.** Effect of aging on hemodynamic response to metaboreflex activation. *Eur J Appl Physiol* 115: 1693–1703, 2015. doi:10.1007/s00421-015-3153-5.
35. **Milia R, Velluzzi F, Roberto S, Palazzolo G, Sanna I, Sainas G, Pusceddu M, Mulliri G, Loviselli A, Crisafulli A.** Differences in hemodynamic response to metaboreflex activation between obese patients with metabolic syndrome and healthy subjects with obese phenotype. *Am J Physiol Heart Circ Physiol* 309: H779–H789, 2015. doi:10.1152/ajpheart.00250.2015.
36. **Moran D, Epstein Y, Keren G, Laor A, Sherez J, Shapiro Y.** Calculation of mean arterial pressure during exercise as a function of heart rate. *Appl Human Sci* 14: 293–295, 1995. doi:10.2114/ahs.14.293.
37. **Murphy MN, Mizuno M, Mitchell JH, Smith SA.** Cardiovascular regulation by skeletal muscle reflexes in health and disease. *Am J Physiol Heart Circ Physiol* 301: H1191–H1204, 2011. doi:10.1152/ajpheart.00208.2011.
38. **Nazir FS, Alem M, Small M, Connell JM, Lees KR, Walters MR, Cleland SJ.** Blunted response to systemic nitric oxide synthase inhibition in the cerebral circulation of patients with Type 2 diabetes. *Diabet Med* 23: 398–402, 2006. doi:10.1111/j.1464-5491.2006.01815.x.
39. **Nishiyasu T, Tan N, Morimoto K, Nishiyasu M, Yamaguchi Y, Murakami N.** Enhancement of parasympathetic cardiac activity during activation of muscle metaboreflex in humans. *J Appl Physiol (1985)* 77: 2778–2783, 1994. doi:10.1152/jappl.1994.77.6.2778.
40. **Nóbrega AC, O'Leary D, Silva BM, Marongiu E, Piepoli MF, Crisafulli A.** Neural regulation of cardiovascular response to exercise: role of central command and peripheral afferents. *BioMed Res Int* 2014: 478965, 2014. doi:10.1155/2014/478965.
41. **O'Leary DS, Augustyniak RA.** Muscle metaboreflex increases ventricular performance in conscious dogs. *Am J Physiol Heart Circ Physiol* 275: H220–H224, 1998. doi:10.1152/ajpheart.1998.275.1.H220.
42. **O'Leary DS, Sala-Mercado JA, Augustyniak RA, Hammond RL, Rossi NF, Ansorge EJ.** Impaired muscle metaboreflex-induced increases in ventricular function in heart failure. *Am J Physiol Heart Circ Physiol* 287: H2612–H2618, 2004. doi:10.1152/ajpheart.00604.2004.
43. **O'Leary DS.** Altered reflex cardiovascular control during exercise in heart failure: animal studies. *Exp Physiol* 91: 73–77, 2006. doi:10.1113/expphysiol.2005.031179.
44. **Olver TD, Laughlin MH.** Endurance, interval sprint, and resistance exercise training: impact on microvascular dysfunction in type 2 diabetes. *Am J Physiol Heart Circ Physiol* 310: H337–H350, 2016. doi:10.1152/ajpheart.00440.2015.
45. **Piepoli MF, Crisafulli A.** Pathophysiology of human heart failure: importance of skeletal muscle myopathy and reflexes. *Exp Physiol* 99: 609–615, 2014. doi:10.1113/expphysiol.2013.074310.
46. **Poitras VJ, Hudson RW, Tschakovsky ME.** Exercise intolerance in type 2 diabetes: is there a cardiovascular contribution? *J Appl Physiol (1985)* 124: 1117–1139, 2018. doi:10.1152/japplphysiol.00070.2017.
47. **Powell BD, Redfield MM, Bybee KA, Freeman WK, Rihal CS.** Association of obesity with left ventricular remodeling and diastolic dysfunction in patients without coronary artery disease. *Am J Cardiol* 98: 116–120, 2006. doi:10.1016/j.amjcard.2006.01.063.
48. **Reaven GM.** Banting Lecture 1988. Role of insulin resistance in human disease. 1988. *Nutrition* 13: 65, 1997.
49. **Roberto S, Crisafulli A.** Consequences of type 1 and 2 diabetes mellitus on the cardiovascular regulation during exercise: a brief review. *Curr Diabetes Rev* 13: 560–565, 2017. doi:10.2174/1573399812666160614123226.
50. **Roberto S, Marongiu E, Pinna M, Angius L, Olla S, Bassareo P, Tocco F, Concu A, Milia R, Crisafulli A.** Altered hemodynamics during muscle metaboreflex in young type 1 diabetes patients. *J Appl Physiol (1985)* 113: 1323–1331, 2012. doi:10.1152/japplphysiol.00280.2012.
51. **Roberto S, Mulliri G, Milia R, Solinas R, Pinna V, Sainas G, Piepoli MF, Crisafulli A.** Hemodynamic response to muscle reflex is abnormal in patients with heart failure with preserved ejection fraction. *J Appl Physiol (1985)* 122: 376–385, 2017. doi:10.1152/japplphysiol.00645.2016.
52. **Russo I, Penna C, Musso T, Popara J, Alloatti G, Cavalot F, Pagliaro P.** Platelets, diabetes and myocardial ischemia/reperfusion injury. *Cardiovasc Diabetol* 16: 71, 2017. doi:10.1186/s12933-017-0550-6.
53. **Sainas G, Milia R, Palazzolo G, Ibba G, Marongiu E, Roberto S, Pinna V, Ghiani G, Tocco F, Crisafulli A.** Mean blood pressure assessment during post-exercise: result from two different methods of calculation. *J Sports Sci Med* 15: 424–433, 2016.
54. **Sala-Mercado JA, Hammond RL, Kim JK, Rossi NF, Stephenson LW, O'Leary DS.** Muscle metaboreflex control of ventricular contractility during dynamic exercise. *Am J Physiol Heart Circ Physiol* 290: H751–H757, 2006. doi:10.1152/ajpheart.00869.2005.
55. **Sanna I, Pinna V, Milia R, Roberto S, Olla S, Mulliri G, Crisafulli A.** Hemodynamic responses during enduro-motorcycling performance. *Front Physiol* 8: 1062, 2017. doi:10.3389/fphys.2017.01062.

56. **Schillaci G, Pirro M, Pucci G, Mannarino MR, Gemelli F, Siepi D, Vaudo G, Mannarino E.** Different impact of the metabolic syndrome on left ventricular structure and function in hypertensive men and women. *Hypertension* 47: 881–886, 2006. doi:10.1161/01.HYP.0000216778.83626.39.
57. **Sheriff DD, Augustyniak RA, O'Leary DS.** Muscle chemoreflex-induced increases in right atrial pressure. *Am J Physiol* 275: H767–H775, 1998. doi:10.1152/ajpheart.1998.275.3.H767.
58. **Shoemaker JK, Mattar L, Kerbeci P, Trotter S, Arbeille P, Hughson RL.** WISE 2005: stroke volume changes contribute to the pressor response during ischemic handgrip exercise in women. *J Appl Physiol (1985)* 103: 228–233, 2007. doi:10.1152/japplphysiol.01334.2006.
59. **Spranger MD, Sala-Mercado JA, Coutsos M, Kaur J, Stayer D, Augustyniak RA, O'Leary DS.** Role of cardiac output versus peripheral vasoconstriction in mediating muscle metaboreflex pressor responses: dynamic exercise versus postexercise muscle ischemia. *Am J Physiol Regul Integr Comp Physiol* 304: R657–R663, 2013. doi:10.1152/ajpregu.00601.2012.
60. **Strom JL, Egede LE.** The impact of social support on outcomes in adult patients with type 2 diabetes: a systematic review. *Curr Diab Rep* 12: 769–781, 2012. doi:10.1007/s11892-012-0317-0.
61. **Thaning P, Bune LT, Hellsten Y, Pilegaard H, Saltin B, Rosenmeier JB.** Attenuated purinergic receptor function in patients with type 2 diabetes. *Diabetes* 59: 182–189, 2010. doi:10.2337/db09-1068.
62. **Vianna LC, Deo SH, Jensen AK, Holwerda SW, Zimmerman MC, Fadel PJ.** Impaired dynamic cerebral autoregulation at rest and during isometric exercise in type 2 diabetes patients. *Am J Physiol Heart Circ Physiol* 308: H681–H687, 2015. doi:10.1152/ajpheart.00343.2014.